weekly up to 100 days post-alloHSCT. Circulating myeloid ($CD11c^{hi}$) and plasmacytoid ($CD123^{hi}$) DCs were enumerated and the expression of CMRF-44 was assessed on $CD11c^{hi}$ DC by four colour flow cytometry. Multivariate analyses were performed using a non-parametric Mann-Whitney *U*-test and receiver operating characteristic (ROC) curves. Following alloHSCT, the severity of acute GVHD correlated with the number of total DC in the blood ($P = .035$). Furthermore, low myeloid and plasmacytoid DC numbers were significantly associated with grade 2-4 acute GVHD ($P = .046$ and .017 respectively). In 40 alloHSCT patients, 27 developed acute GVHD. CMRF-44 was expressed on $CD11c^{+}$ DC in all cases prior to the onset of acute GVHD. Of the 13 patients without GVHD, 8 had no circulating CMRF-44^{+} $CD11c^{+}$ DC. CMRF-44 expression was independent of the reconstitution of myeloid DC ($P = .73$). Patients who had CMRF-44^{+} $CD11c^{+}$ DC in more than 20% of their post-transplant monitoring samples were more likely to develop acute GVHD ($P = .001$, OR = 37.1). In addition, patients with more severe grade 2-4 GVHD had significantly higher percentages of CMRF-44^{+} $CD11c^{+}$ DCs ($P = .001$). CMRF-44 expression at greater than or equal to 12% of $CD11c^{hi}$ DCs had a sensitivity of 87.5 for prediction of grade 2-4 acute GVHD and a specificity of 91.7. We conclude that CMRF-44 expression on blood $CD11c^{+}$ DC is highly associated with the onset of acute GVHD. The detection of circulating activated DCs may be used as a predictive tool to identify patients at risk of severe GVHD and potentially to direct therapy. Our data also reinforces the potential for suitably engineered CMRF-44 or other antibodies directed at DC activation antigens to prevent or treat GVHD.

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DETERMINING PROGNOSIS FOR PATIENTS WITH ACUTE GVHD IN REAL TIME: DEVELOPMENT AND TESTING OF AN ACUTE GVHD ACTIVITY INDEX

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The current scoring systems for acute GVHD are problematic due to the retrospective assignment of scores, lack of consideration for the efficacy of treatment, and inter-observer error. An evidence-based, real-time system for scoring GVHD would be useful for determining prognosis in individual patients and for quantifying the burden of GVHD across time. **Methods:** We examined the severity of signs and symptoms of GVHD from its onset to day 100 in 386 patients with CML who underwent allogeneic transplant after a myeloablative regimen. During each 10 day period to day 100, a unique letter designation was assigned for each degree of abnormality in skin, liver, upper gastrointestinal tract, lower gastrointestinal tract, immunosuppressive drugs, fever, and performance status, respectively. We used a training data set of 193 randomly selected patients, logistic regression methods, and receiver-operator curves for optimizing the model, to create an activity index (aGVHDAI) that predicts non-relapse mortality at day 200. Scaling was carried out by dividing the coefficients in the logistic regression model by the sum of the coefficients and multiplying this proportion by 100. The final aGVHDAI was then applied to an independent, 193-patient data set, testing the accuracy of the index to predict day 200 non-relapse mortality. **Results:** Parameters entering the optimal aGVHDAI model included the level of jaundice, caloric intake, need for prednisone therapy, and performance score. Skin GVHD, volume of diarrhea, and presence of fever did not improve the model's predictive ability (Table 1). The area under a receiver-operator curve for the final average aGVHDAI model in the training data set, using non-relapse mortality at day 200 as the end point, was 0.87. When the model was then applied to an independent data set of 193 patients, the area under an ROC curve was 0.85. Using the same aGVHD activity index parameters, a graphic was developed that allows prediction of non-relapse mortality at day 200 (on the vertical axis) for individual patients, based on the aGVHDAI at any point in time after transplant (on the horizontal axis). **Conclusions:** An acute GVHD activity index has been tested that allows prediction of non-relapse mortality in real-time and provides investigators with a research tool for assessing the burden of acute GVHD over time. This aGVHDAI, scaled from 0-100, is based on serum bilirubin levels, caloric intake, need for prednisone therapy, and performance status.

Table 1. *Components of the Acute GVHD Activity Index with Weighting Factors for Each component*

Factor	Scoring Level	Scaled Weighting Factor
Liver dysfunction	Total serum bilirubin 2–4 mg/dL	16
	Total serum bilirubin ≥5 mg/dL	26
Upper GI tract	Oral caloric intake <40%, with poorly controlled anorexia, nausea, or vomiting	20
Immunosuppressive therapy	Any prednisone dose or secondary therapy for GVHD	17
Performance status	Ambulatory but restricted in strenuous activity	20
	Limited self-care, more than 50% of time in bed, or worse	37

The scale is from 0 to 100, with the end point of day 200 non-relapse mortality.

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THE CYTOKINE STORM AND ACUTE GRAFT-VERSUS-HOST DISEASE (aGVHD) AFTER REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (allo-SCT)

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The use of RIC regimens has modified the natural history of transplant-related complications, especially aGVHD. The aim of this study was to investigate the role of inflammatory cytokines on aGVHD incidence and severity in 113 patients who received a RIC allo-SCT from an HLA-identical sibling. Plasma levels of 10 different cytokines (IL-1beta, IL-6, IL-8, IL-10, IL-12p70, IL-18, TNF-α, IFN-α, IFN-γ, and Fas-L) were measured by ELISA prior to allo-SCT, at day 0 prior to graft infusion, and at regular times within the first 3 months after allo-SCT. Except for IL-12p70, all measured cytokines showed little variations in the blood in the first three months after allo-SCT. The incidence of grade II-IV aGVHD was 45% (95% CI, 36-54%; median onset, 32 days after allo-SCT). In the subgroup of patients for whom all tested cytokines could be measured closely, but rigorously prior to aGVHD clinical onset, a high IL-12p70 level ($P < 10^{-4}$) was significantly associated with the development of clinically significant grade II-IV aGVHD. IL-12p70 levels were significantly correlated to the severity of aGVHD: grade 0-I, median 468 pg/ml; grade II, median 2538 pg/ml; and grade III-IV, median 4615 pg/mL ($P = .0001$). In patients experiencing grade II-IV aGVHD, IL-12p70 levels decreased after aGVHD therapy. Interestingly, we found a more rapid recovery of monocytes, the main pool of IL-12p70-secreting myeloid dendritic cells (DC), prior to aGVHD clinical onset in patients with grade II-IV aGVHD, as compared to patients with grade 0-I aGVHD (median, 829/μL vs. 552/μL; $P = .005$). At the effector level, we observed a significantly more robust recovery of genuine naive CD3+CD4+CD45RA+CD27+ T cells prior to aGVHD clinical onset, in patients with grade II-IV aGVHD, as compared to patients with grade 0-I aGVHD (median, 50/μL vs. 16/μL; $P = .006$). In multivariate analysis, IL-12p70 level measured before aGVHD clinical onset was the strongest predictive factor for aGVHD development and severity ($P < 10^{-4}$; RR = 10.7; 95% CI, 3.8-30.6). Overall, these findings reconstitute a genuine Th1 loop, supporting a model where aGVHD primarily reflects a type 1 alloreaction (rapid monocytes/DC recovery, IL-

12p70 secretion, naive CD4+ T cell expansion, Th1 and Tc1 cells differentiation) in the context of RIC allo-SCT. The fine functions of immune effectors would tend to be more evident in such less toxic regimens, offering new opportunities for a better understanding of aGVHD pathophysiology and therapy.

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RAPAMYCIN ADDED TO HUMAN CD25+ CELL CULTURES ACTIVATED THROUGH CD3/CD28 ENRICHES FOR CD4+CD25+CD27+Foxp3+ REGULATORY T CELLS

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CD4+ T cells that constitutively express high levels of CD25 inhibit T-cell responses in a dose-dependent manner. Methods to isolate and expand CD4+CD25+ regulatory T-cells (Tregs) have been developed to allow for potential clinical use in regulating undesired T cell-mediated responses such as autoimmune disease or GVHD. We have enriched for Tregs by CD25 positive selection followed by culture in ex vivo 15-5% HS and autologous feeder cells. Activation is through a single addition of CD3/CD28 Ab-linked beads (4:1 bead:cell) and IL-2 (100 U/mL added d 3). Cultures with potent suppressor activity expanded on average 450 fold in 14 d when maintained at 5×10^5/mL in medium + IL-2. However, by 21 d suppressor activity greatly diminished and the % of CD25-bright cells decreased, indicating activated non-Treg cells were dominating the cultures. To increase the purity of Tregs we explored the use of rapamycin, a T cell immunosuppressive agent recently shown to selectively spare murine Tregs. Rapamycin (20-0.01 ng/mL) added to CD25-enriched cells significantly inhibited cell expansion at doses $\geq$1 ng/mL that was inversely correlated with enhanced suppressor activity at days 10 and 21. Cultures without rapamycin expanded on average 17,000 fold by day 21 vs 350 fold with 1 ng/mL rapamycin. By 21 d cells from untreated cultures suppressed allo-proliferation by only 28% at a 1:1 suppressor:responder ratio compared to 63 $\pm$ 20% by cells grown in $\geq$1 ng/mL rapamycin (P = .007). The % CD4+CD25bright cells at 10 d and 21 d was higher in rapamycin-containing cultures than untreated cultures, $P < .003$. Intracellular FACS staining for the transcription factor Foxp3, a marker for Tregs, showed significantly higher expression on CD4+ cells from 10 d rapamycin containing cultures with 23 $\pm$ 6% Foxp3+CD4+ cells vs 5 $\pm$ 3% in cultures without rapamycin (P = .03, n = 4). Foxp3 was predominately expressed on CD4+CD25-bright cells that also co-expressed CD27-bright. By day 21 cultures grown in rapamycin contained 1000 to 5000 fold more CD4+Foxp3+ cells than 10 d cultures without rapamycin. Only 0.7% of CD4+ cells from CD4+CD25-neg cells cultured with rapamycin expressed Foxp3. Optimal Foxp3 expression required continued presence of rapamycin in culture and addition at culture initiation was superior to addition at day 3. In summary, addition of rapamycin at doses from 1-20 ng/mL to CD25-enriched cell cultures increased the purity of cells with the phenotype and function of Tregs.

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PROSPECTIVE EVALUATION OF A GvHD-SPECIFIC PROTEOMICS PATTERN AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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We have recently published a polypeptide pattern specific for the early diagnosis of acute graft versus host disease (aGvHD), based on the application of capillary electrophoresis (CE) and mass spectrometry (MS). Here we report the application of aGvHD-specific patterns to prospectively collected samples from 86 patients (45 AML, 14 ALL, 8 MM, 5 CLL, 5 SAA, 3 MDS, 3CML, 3 NHL). Fifty-three patients were transplanted from matched unrelated donors (MUD), 29 received stem cells from matched related donors (MRD), 3 from haplo-identical donors and 1 was transplanted from a syngeneic sibling. In the majority of the patients the GvHD prophylaxis was methotrexate or mycophenolate and cyclosporin A. Urine samples were collected on ice prior to conditioning, weekly until discharge from the ward and monthly thereafter. Immediate freezing of the samples avoids degradation of the proteins/peptides. After thawing and removal of confounding substances like salts and of all molecules larger than 30 kDa, the samples were loaded onto the CE, separated according to their charge and, after ionization, directly analyzed in an electrospray-ionization time-of-flight (ESI-TOF)-MS. Between 500 and 2500 peptides and proteins were detected in individual samples. All data generated are stored in a Microsoft MS database. The polypeptide patterns specific for the early detection of acute GvHD were applied to the data from the prospectively and blinded collected samples. The outcome of these analyses was compared to the clinical diagnosis of aGvHD, sepsis and CMV-reactivation. In 362 samples screened, 112 scored positive with the GvHD pattern. Twenty-eight of those were false positive, mainly at the time of conditioning, but only 3 were scored false negative. Thus the sensitivity of the aGvHD pattern is about 96% with a 75% positive prediction value, the specificity is about 89% with a negative prediction value of 98%. Thus the application of the aGvHD pattern for early recognition of acute GvHD is very useful for predicting the development of aGvHD. Seven patients in the prospective cohort have developed cGvHD so far and samples were also scored with the aGvHD pattern. First results show that in the majority of the patients the polypeptides excreted do not correspond to those forming the aGvHD pattern. Taken together our results demonstrate that the proteome analysis of body fluids collected from patients after HSCT maybe extremely useful for diagnosis of complications.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS: RESULTS OF THE PROSPECTIVE, MULTI-INSTITUTIONAL CORD BLOOD TRANSPLANTATION STUDY (COBLT)

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In 1996, NHLBI sponsored COBLT, a prospective study that established 3 cord blood banks that banked ~8000 CBUs, and funded 28 transplant centers to participate in a clinical trial with the primary endpoint of 180-day post-transplant overall survival (OS). The trial enrolled 316 pediatric pts with median age 4.6 years (range 0.1-17.9), 61% M, 37% minorities, 44% CMV+ and 71% with a malignant (malign) disease, who received a single CBU. CBUs had to be at least 4 of 6 HLA match (intermediate resolution A and B and high resolution [HR] DRB1) and provide a total nucleated cell dose (TNC) $\geq 1.0 \times 10^7$/kg. Approximately 50% of Caucasian and Hispanic pts received a CBU matched at HLA 5-6/6, but only 15% of African-Americans and 36% of Asians were matched at HLA 5-6/6. HR HLA types were retrospectively determined for HLA -A, -B, -DRB1 (292 pairs) and -C and -DQB1 (270 pairs). Only 30% of pairs were HR HLA matched at 8-10 alleles. The median pre-cryopreservation TNC and CD34+ cell dose were 6.8×10^7/kg (range 1.5-50.4) and 2.3×10^5/kg (range 0.1-20.1), respectively. The cumulative incidence (CINC) of neutrophil recovery (>500, day 42) and of platelet engraftment (>50000, day 180) was 81% and 54%, respectively. By day +100, the CINC of grades II-IV aGVHD was 40%. The CINC of cGVHD was 21% at 2 yrs. The probability of OS at 180 days